Supporting Information

Novel Carboline Derivatives as Potent Antifungal Lead Compounds: Design, Synthesis and Biological Evaluation

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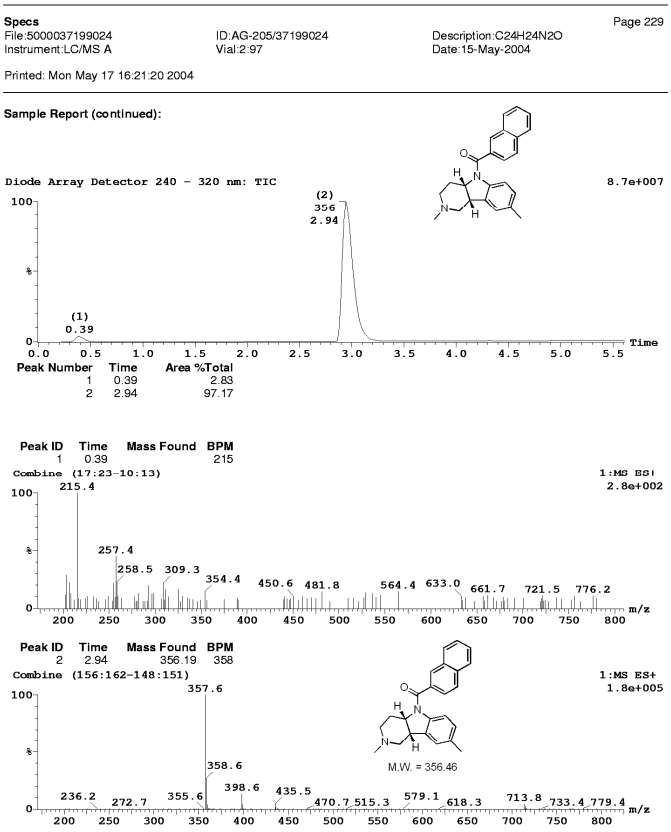
1. Synthetic procedures of the target compounds

Chemistry. General Methods. All reagents and solvents were reagent grade or were purified by standard methods before use. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on Bruker AVANCE300, AVANCE500, or AVANCE600 spectrometer (Bruker Company, Germany), using TMS as an internal standard and $CDCl_3$ or DMSO-d_6 as solvents. Chemical shifts (δ values) and coupling constants (J values) are given in ppm and Hz, respectively. Elemental analyses were performed with a MOD-1106 instrument and were consistent with theoretical values within 0.4%. The mass spectra were recorded on an Esquire 3000 LC-MS mass spectrometer. TLC analysis was carried out on silica gel plates GF254 (Qindao Haiyang Chemical, China). Silica gel thin-layer chromatography was performed on precoated plates GF-254 (Qingdao Haiyang Chemical, China). Purity of the compounds was analyzed by HPLC using 30:70 MeOH/H_2O as the mobile phase with a flow rate of 0.8 mL/min on a C18 column (Aglilent 20RBA × SB-C18, 5 μ m, 4.6 mm × 150 mm). All compounds exhibited greater than 95% purity.

Compound **1** was obtained from the Specs Database (www.specs.net). The compound ID is AG-205/37199024 and related spectrum was listed as follows.



Chemical synthesis of 5-benzyl-8-chloro-2-methyl-2,3,4,5-tetrahydro-1*H*-pyrido [4,3-*b*]indole (C1)

A solution of 8-chloro-2-methyl-2,3,4,5-tetrahydro-1*H*-pyrido[4,3-*b*]indole[1] (**4**, 0.22 g, 1 mmol) and NaH (0.036 g, 1.5 mmol) in 20 mL DMF was stirred at room temperature for 15 min. Then, benzyl bromide (0.17 g, 1 mmol) was added and stirred for 6 h. After reaction, the solution was diluted by H_2O (40 mL) and extracted by EtOAc (50 mL × 3). The organic layer was combined, dried with Na_2SO_4, and concentrated under reduced pressure. The residue was purified by column

chromatography (CH_2Cl_2: MeOH = 100: 1) to give compound **C1** as yellow solid (0.19 g, 62.4%). ^{1}H-NMR δ: 2.55 (s, 3H), 2.76-2.82 (m, 4H), 3.65 (s, 2H), 5.20 (s, 2H), 6.94 (m, 2H), 7.03 (d, J = 8.4 Hz, 1H), 7.08 (d, J = 8.4 Hz, 1H), 7.21-7.25 (m, 3H),7.39 (s, 1H). ^{13}C-NMR ($CDCl_3$, 150 MHz) δ: 22.91, 45.62, 46.72, 51.61, 52.38, 110.38 117.40, 121.35, 125.09, 126.19 (2C), 127.62, 128.97 (2C), 134.95, 135.40, 137.46. MS (ESI, positive) m/z calcd for $C_{19}H_{20}ClN_2$ (M+H): 311.13; found 311.01. Anal. calcd. for $C_{19}H_{19}ClN_2$: C, 73.42; H, 6.16; N, 9.01. Found: C, 73.41; H, 6.15; N, 9.01. HPLC purity: 97.2%.

The synthetic method for compounds **C2-C6 and C8-C10** was similar to the synthesis of compound **C1**.

Chemical synthesis of 2-(8-chloro-2-methyl-3,4-dihydro-1*H*-pyrido[4,3-*b*]indol -5(2*H*)-yl)-1-phenylethanol (C7)

A solution of Intermediate **4** (0.10 g, 0.45 mmol) and NaH (0.029 g) in 15 mL DMF was stirred at room temperature for 15 min. Then, 2-phenyloxirane (0.16 g, 1.35 mmol) was added and stirred at 45°C for overnight. After reaction, the solution was diluted by H_2O (40 mL) and extracted by EtOAc (50 mL×3). The organic layer was combined, dried with Na_2SO_4, and concentrated under reduced pressure. Then the residue was purified by column chromatography (CH_2Cl_2: MeOH = 100: 3) to give compound **C7** as white solid 0.10 g (65.3%). ^{1}H-NMR ($CDCl_3$, 600 MHz) δ: 2.48 (s, 3H), 2.63 (m, 1H), 2.78 (m, 2H), 2.86 (m, 1H), 3.54 (s, 2H), 4.07-4.15 (m, 2H), 4.84 (m, 1H), 7.07 (dd, J_1 = 1.8 Hz, J_2 = 8.4 Hz, 1H), 7.18 (d, J = 8.4 Hz, 1H), 7.17 (d, J = 9.0 Hz, 1H), 7.25-7.30 (m, 6H). ^{13}C-NMR ($CDCl_3$, 150 MHz) δ: 22.88, 45.22, 51.34,

51.45, 52.34, 73.56, 106.99, 110.57, 117.23, 121.08, 124.87, 125.88 (2C), 126.54, 128.14, 128.70 (2C), 135.30, 135.35, 142.35. MS (ESI, positive) m/z calcd for $C_{20}H_{22}ClN_2O$ (M+H): 341.14; found 341.09. Anal. calcd. for $C_{20}H_{21}ClN_2O$: C, 70.48; H, 6.21; N, 8.22. Found: C, 70.47; H, 6.20; N, 8.23. HPLC purity: 98.2%.

Chemical synthesis of 6-chloro-5-(3-(4-chlorophenoxy)propyl)-2-methyl-2,3,4,5-tetrahydro-1H-pyrido[4,3-b]indole (C11)

To a solution of 4-chlorophenol (6.43 g, 0.05 mol) and K_2CO_3 (6.9 g, 0.05 mol) in 50 mL EtOH, 1,3-dibrompropane (21.09 g, 0.10 mol) was added dropwise and stirred at 80°C for 4 h. After reaction, the solvent was removed under reduced pressure, diluted with H_2O (70 mL) and extracted by EtOAc (80 mL×3). Then, the organic layer was separated, dried with Na_2SO_4, and concentrated under reduced pressure. The residue was purified by column chromatography (hexane) to give 1-(3-bromopropoxy)-4-chlorobenzene as transparent oil: 9.89 g (79.3%). ^{1}H-NMR ($CDCl_3$, 600 MHz) δ: 2.30 (m, 2H), 3.59 (t, J = 6.0 Hz, 2H), 4.07 (t, J = 6.0 Hz, 2H), 6.83 (d, 2H), 7.22 (d, 2H). ^{13}C-NMR ($CDCl_3$, 150 MHz) δ: 29.77, 32.20, 65.60, 116.00, 125.75, 129.30, 157.26. MS (ESI, positive) m/z calcd for $C_9H_{11}BrClO$ (M+H): 248.97; found 249.06.

A solution of Intermediate **4** (0.10 g, 0.45 mmol) and KOH (0.1 g, 1.8 mmol) in 20 mL DMSO was stirred at room temperature for 15 min. Then, 1-(3-bromopropoxy)-4-chlorobenzene (0.11 g, 0.45 mmol) was added and stirred at room temperature for overnight. Then, the reaction was diluted by H_2O (40 mL) and extracted by EtOAc (50 mL × 3). The organic layer was separated, dried with Na_2SO_4,

and concentrated under reduced pressure. The residue was purified by column chromatography (CH_2Cl_2: MeOH = 100: 1) to give the target compound **C11** as yellow oil (0.10 g, 57.1%). ^{1}H-NMR ($CDCl_3$, 600 MHz) δ: 2.54 (m, 2H), 2.55 (s, 3H), 2.80 (t, J = 5.4 Hz, 2H), 2.86 (t, J = 5.4 Hz, 2H), 3.66 (s, 2H), 3.90 (t, J = 6.0 Hz, 2H), 4.59 (t, J = 7.2 Hz, 2H), 6.79-6.81 (d, J = 9.0 Hz, 2H), 6.98 (t, J = 7.8 Hz, 1H), 7.11 (d, J = 8.0 Hz, 1H), 7.23-7.24 (d, J = 9.0 Hz, 2H), 7.27 (d, J = 8.0 Hz, 1H). ^{13}C-NMR ($CDCl_3$, 150 MHz) δ: 22.40, 31.83, 41.32, 45.05, 51.29, 52.25, 64.87, 115.68, 116.16, 116.32, 119.96, 123.07, 125.79, 128.90, 129.38, 129.56, 131.63, 134.72, 157.16. MS (ESI, positive) m/z calcd for $C_{21}H_{23}Cl_2N_2O$ (M+H): 389.12; found 389.13. Anal. calcd. for $C_{21}H_{22}Cl_2N_2O$: C, 64.79; H, 5.70; N, 7.20. Found: C, 64.77; H, 5.69; N, 7.21. HPLC purity: 96.1%. The synthetic method for compounds **C12-C18** was similar to the synthesis of compound **C11**.

Chemical synthesis of 5-(3-(4-chlorophenoxy)propyl)-8-fluoro-2,3,4,5-tetrahydro -1H-pyrido[4,3-b]indole (C38)

To a solution of ethyl 8-fluoro-3,4-dihydro-1H-pyrido[4,3-b]indole-2(5H)-carboxylate (**6**, 0.10 g, 0.38 mmol) in 10 mL DMSO, KOH (21 mg, 0.38 mmol) was added and stirred at room temperature for 15 min. Then, 1-(3-bromopropoxy)-4-chlorobenzene (**7**, 78 mg, 0.38 mmol) was added and stirred at room temperature for overnight. After reaction, the solution was diluted with H_2O (50 mL) and extracted by EtOAc (40 mL×3). Then, the organic layer was separated, dried with Na_2SO_4, and concentrated under reduced pressure. The residue was purified by column chromatography (PE: EtOAc = 5: 1) to give ethyl 5-(3-(4-chlorophenoxy)propyl)-

8-fluoro-3,4-dihydro-1H-pyrido[4,3-b]indole-2(5H)-carboxylate (intermediate **8**) as yellow oil 0.12 g (73.4%). ^{1}H-NMR (300 Hz, $CDCl_3$) δ: 1.30 (t, J = 7.2 Hz, 3H), 2.20 (m, 2H), 2.79 (br, 2H), 3.71-3.87 (br, 4H), 4.19 (q, J = 7.2 Hz, 2H), 4.26 (t, J = 6.7 Hz, 2H), 4.59-4.69 (br, 2H), 6.78 (d, J = 8.8 Hz, 2H), 6.87 (d, J = 9.1 Hz, 1H), 7.10 (d, J = 9.4 Hz, 1H), 7.18 (s, 1H), 7.26 (d, J = 8.8 Hz, 2H). ^{13}C-NMR ($CDCl_3$, 75 MHz) δ: 14.86, 29.78, 39.65, 41.22, 41.23, 61.72, 64.43, 109.28, 109.63, 109.70, 109.82, 115.75 (2C), 115.67, 126.09, 129.58 (2C), 133.08, 156.30, 157.13, 159.41. MS (ESI, positive) m/z calcd for $C_{23}H_{25}ClFN_2O_3$ (M+H): 431.15; found 431.12. Anal. calcd. for $C_{23}H_{24}ClFN_2O_3$: C, 64.11; H, 5.61; N, 6.50. Found: C, 64.12; H, 5.62; N, 6.50.

To a solution of intermediate **8** (0.20 g, 0.47 mmol) in 5 mL EtOH, Claisen hydrolysate (KOH, 1.2 g; H_2O, 0.5 mL; EtOH, 5 mL) was added and stirred at 80℃ for 3h. After reaction, the solvent was removed under reduced pressure, and the residue was purified by column chromatography (CH_2Cl_2: MeOH = 100: 5) to give compound **C38** as yellow oil 0.10 g (60.1%). ^{1}H-NMR (500 Hz, $CDCl_3$) δ: 2.19 (m, 2H), 2.79 (t, J = 5.6 Hz, 2H), 3.23 (t, J = 5.7 Hz, 2H), 3.81(t, J = 6.6 Hz, 2H), 4.07 (s, 2H), 4.23 (t, J = 5.5 Hz, 2H), 6.78～7.26 (m, 7H). ^{13}C-NMR ($CDCl_3$, 75 MHz) δ: 22.15, 29.66, 39.50, 41.55, 42.70, 64.35, 102.75, 103.06, 109.26, 109.59, 109.71, 115.70 (2C), 125.59, 129.43, 129.47 (2C), 132.76, 134.46, 157.02. MS (ESI, positive) m/z calcd for $C_{20}H_{21}ClFN_2O$ (M+H): 359.13; found 359.07. Anal. calcd. for $C_{20}H_{20}ClFN_2O$: C, 66.94; H, 5.62; N, 7.81. Found: C, 66.96; H, 5.63; N, 7.82. HPLC purity: 96.5%. The synthetic method for compounds **C19-C37 and C39-C42** was similar to the synthesis of compound **C38**.

2. Structural characterization of the target compounds

8-chloro-5-(2-chlorobenzyl)-2-methyl-2,3,4,5-tetrahydro-1*H*-pyrido[4,3-*b*]indole (C2). Brown solid, 0.15 g (59.8%). ^{1}H-NMR ($CDCl_3$, 600 MHz) δ: 2.57 (s, 3H, NC$\underline{H_3}$), 2.84-2.86 (m, 4H, ArC$\underline{H_2}$C$\underline{H_2}$N), 3.68 (s, 2H, ArC$\underline{H_2}$NCH$_3$), 5.18 (s, 2H, ArC$\underline{H_2}$N), 6.78 (d, J = 7.2 Hz, 1H, Ar-H), 7.02 (s, 1H, Ar-H), 7.06 (s, 1H, Ar-H), 7.17 (t, J = 7.8 Hz, 1H, Ar-H), 7.21 (d, J = 8.4 Hz, 1H, Ar-H), 7.40 (s, 1H, Ar-H). MS (ESI, positive) m/z calcd for $C_{19}H_{19}Cl_2N_2$ (M+H): 345.09; found 345.01. Anal. calcd. for $C_{19}H_{18}Cl_2N_2$: C, 66.09; H, 5.25; N, 8.11. Found: C, 66.07; H, 5.25; N, 8.12. HPLC purity: 97.2%.

8-chloro-5-(3-chlorobenzyl)-2-methyl-2,3,4,5-tetrahydro-1*H*-pyrido[4,3-*b*]indole (C3). Brown solid, 0.14 g (64.8%). ^{1}H-NMR ($CDCl_3$, 600 MHz) δ: 2.54 (s, 3H, NC$\underline{H_3}$), 2.72-2.81 (m, 4H, ArC$\underline{H_2}$C$\underline{H_2}$N), 3.63 (s, 2H, ArC$\underline{H_2}$NCH$_3$), 5.14 (s, 2H, ArC$\underline{H_2}$N), 6.84 (d, J = 8.4 Hz, 2H, Ar-H), 7.02(s, 2H, Ar-H), 7.18 (d, J = 8.4 Hz, 2H, Ar-H), 7.39 (s, 1H, Ar-H). ^{13}C-NMR ($CDCl_3$, 75 MHz) δ: 23.06, 45.80, 46.08, 51.61, 52.40, 108.54, 110.19, 117.51, 121.41, 125.20, 126.91, 127.54 (2C), 129.12 (2C), 133.43, 134.91, 135.21, 135.99. MS (ESI, positive) m/z calcd for $C_{19}H_{19}Cl_2N_2$ (M+H): 345.09; found 345.11. Anal. calcd. for $C_{19}H_{18}Cl_2N_2$: C, 66.09; H, 5.25; N, 8.11. Found: C, 66.08; H, 5.26; N, 8.12. HPLC purity: 96.1%.

(2-bromophenyl)(8-chloro-2-methyl-3,4-dihydro-1*H*-pyrido[4,3-*b*]indol-5(2*H*)-yl) methanone (C4). White solid, 0.21 g (63.7%). ^{1}H-NMR ($CDCl_3$, 600 MHz) δ: 2.53 (s, 2H, ArCH$_2$C$\underline{H_2}$N), 2.54 (s, 3H, NC$\underline{H_3}$), 2.69 (s, 2H, ArC$\underline{H_2}$CH$_2$N), 3.61 (s, 2H, ArC$\underline{H_2}$N), 7.15-7.45 (m, 7H, Ar-H). ^{13}C-NMR ($CDCl_3$, 150 MHz) δ: 26.09, 29.68,

45.52, 51.04, 52.50, 116.64, 117.35, 120.02, 124.38, 127.86, 129.02, 129.45, 129.88, 132.01, 133.38, 134.32, 134.72, 138.05, 166.69. MS (ESI, positive) m/z calcd for $C_{19}H_{17}BrClN_2O$ (M+H): 403.02; found 403.12. Anal. calcd. for $C_{19}H_{16}BrClN_2O$: C, 56.53; H, 3.99; N, 6.94. Found: C, 56.54; H, 4.00; N, 6.93. HPLC purity: 96.0%.

(8-chloro-2-methyl-3,4-dihydro-1*H*-pyrido[4,3-*b*]indol-5(2*H*)-yl)(3-chlorophenyl) methanone (C5). White solid, 0.16 g (68.9%). ^{1}H-NMR ($CDCl_3$, 600 MHz) δ: 2.53 (s, 3H, NC$\underline{H}_3$), 2.61-2.69 (m, 4H, ArC$\underline{H}_2$C$\underline{H}_2$N), 3.59 (s, 2H, ArC$\underline{H}_2$N), 7.10-7.59 (m, 7H, Ar-H). ^{13}C-NMR ($CDCl_3$, 75 MHz) δ: 26.90, 45.72, 51.22, 52.55, 116.21, 116.42, 117.62, 123.97, 127.42, 129.06, 129.22, 129.55, 130.17, 132.72, 134.90, 135.13, 137.10, 167.50. MS (ESI, positive) m/z calcd for $C_{19}H_{17}Cl_2N_2O$ (M+H): 359.07; found 359.14. Anal. calcd. for $C_{19}H_{16}Cl_2N_2O$: C, 63.52; H, 4.49; N, 7.80. Found: C, 63.51; H, 4.47; N, 7.81. HPLC purity: 97.4%.

(8-chloro-2-methyl-3,4-dihydro-1*H*-pyrido[4,3-*b*]indol-5(2*H*)-yl)(4-chlorophenyl) methanone (C6). White solid, 0.21 g (66.9%). ^{1}H-NMR ($CDCl_3$, 600 MHz) δ: 2.53 (s, 3H, NC$\underline{H}_3$), 2.66 (m, 4H, ArC$\underline{H}_2$C$\underline{H}_2$N), 3.58 (s, 2H, ArC$\underline{H}_2$N), 7.09 (dd, J_1 = 1.8 Hz, J_2= 9.0 Hz, 1H, Ar-H), 7.32 (d, J = 1.8 Hz, 1H, Ar-H), 7.34 (d, J = 9.0 Hz, 1H, Ar-H), 7.47 (d, J = 8.4 Hz, 2H, Ar-H), 7.61 (d, J = 8.4 Hz, 2H, Ar-H). MS (ESI, positive) m/z calcd for $C_{19}H_{17}Cl_2N_2O$ (M+H): 359.07; found 359.11. Anal. calcd. for $C_{19}H_{16}Cl_2N_2O$: C, 63.52; H, 4.49; N, 7.80. Found: C, 63.50; H, 4.49; N, 7.81. HPLC purity: 95.3%.

1-(8-chloro-2-methyl-3,4-dihydro-1*H*-pyrido[4,3-*b*]indol-5(2*H*)-yl)-2-phenylethan one (C8). Brown solid, 0.10 g (42.3%). ^{1}H-NMR ($CDCl_3$, 600 MHz) δ: 2.54 (s, 3H,

NC$\underline{H_3}$), 2.79 (t, J = 5.8 Hz, 2H, ArCH$_2$C$\underline{H_2}$N), 3.17 (t, 2H, ArC$\underline{H_2}$CH$_2$N), 3.57 (s, 2H, ArC$\underline{H_2}$N), 4.24 (s, 2H, ArC$\underline{H_2}$CO), 7.21-8.07 (m, 8H, Ar-H). MS (ESI, positive) m/z calcd for $C_{20}H_{20}ClN_2O$ (M+H): 339.13; found 339.19. Anal. calcd. for $C_{20}H_{19}ClN_2O$: C, 70.90; H, 5.65; N, 8.27. Found: C, 70.89; H, 5.64; N, 8.28. HPLC purity: 98.6%.

8-chloro-2-methyl-5-(prop-2-yn-1-yl)-2,3,4,5-tetrahydro-1*H*-pyrido[4,3-*b*]indole (C9). Brown solid, 0.15 g (55.7%). ^{1}H-NMR ($CDCl_3$, 600 MHz) δ: 2.28 (s, 1H, C≡C$\underline{H}$), 2.89-2.93 (m, 4H, ArC$\underline{H_2}$C$\underline{H_2}$N), 3.65 (s, 2H, ArC$\underline{H_2}$N), 4.74 (s, 2H, C$\underline{H_2}$C≡CH), 7.14 (dd, J_1 = 1.8 Hz, J_2 = 9.0 Hz, 1H, Ar-H), 7.27 (d, J = 9.0 Hz, 1H, Ar-H), 7.37 (d, J = 1.8 Hz, Ar-H). ^{13}C-NMR ($CDCl_3$, 150 MHz) δ: 22.74, 29.83, 32.59, 45.66, 51.47, 52.28, 72.78, 77.93, 108.81, 110.11, 117.54, 121.46, 125.43, 127.17, 134.49, 134.80. MS (ESI, positive) m/z calcd for $C_{15}H_{16}ClN_2$ (M+H): 259.10; found 258.98. Anal. calcd. for $C_{15}H_{15}ClN_2$: C, 69.63; H, 5.84; N, 10.83. Found: C, 69.62; H, 5.83; N, 10.84. HPLC purity: 97.7%.

2-(8-chloro-2-methyl-3,4-dihydro-1*H*-pyrido[4,3-*b*]indol-5(2*H*)-yl)acetonitrile (C10). Brown solid, 0.13 g (46.7%). ^{1}H-NMR ($CDCl_3$, 600 MHz) δ: 2.58 (s, 3H, NC$\underline{H_3}$), 2.89 (s, 4H, ArC$\underline{H_2}$C$\underline{H_2}$N), 3.62 (s, 2H, ArC$\underline{H_2}$N), 4.87 (s, 2H, C$\underline{H_2}$CN), 7.18-7.21 (m, 2H, Ar-H), 7.39 (d, J = 1.2 Hz, Ar-H). MS (ESI, positive) m/z calcd for $C_{14}H_{15}ClN_3$ (M+H): 260.10; found 260.04. Anal. calcd. for $C_{14}H_{14}ClN_3$: C, 64.74; H, 5.43; N, 16.18. Found: C, 64.75; H, 5.43; N, 16.19. HPLC purity: 96.2%.

5-(3-(4-chlorophenoxy)propyl)-2,7-dimethyl-2,3,4,5-tetrahydro-1*H*-pyrido[4,3-*b*]indole (C12). Yellow solid, 0.16 g (61.5%). ^{1}H-NMR ($CDCl_3$, 600 MHz) δ: 2.18 (m, 2H, NCH$_2$C$\underline{H_2}$CH$_2$O), 2.34 (s, 3H, NC$\underline{H_3}$), 2.60 (s, 3H, ArC$\underline{H_3}$), 2.88 (m, 4H,

ArC$\underline{H_2}$C$\underline{H_2}$N), 3.78 (s, 2H, ArC$\underline{H_2}$N), 3.80 (t, J = 5.8 Hz, 2H, NC$\underline{H_2}$CH$_2$CH$_2$O), 4.22 (t, J = 6.6 Hz, 2H, OC$\underline{H_2}$), 6.78-7.26 (m, 7H, Ar-H). MS (ESI, positive) m/z calcd for $C_{22}H_{26}ClN_2O$ (M+H): 369.17; found 369.10. Anal. calcd. for $C_{22}H_{25}ClN_2O$: C, 71.63; H, 6.83; N, 7.59. Found: C, 71.62; H, 6.83; N, 7.60. HPLC purity: 96.4%.

5-(3-(4-chlorophenoxy)propyl)-2-methyl-8-(trifluoromethyl)-2,3,4,5-tetrahydro-1*H*-pyrido[4,3-*b*]indole (C13). Yellow oil, 0.17 g (63.9%). ^{1}H-NMR ($CDCl_3$, 600 MHz) δ: 2.21 (m, 2H, NCH$_2$C$\underline{H_2}$CH$_2$O), 2.68 (s, 3H, NC$\underline{H_3}$), 3.00 (m, 4H, ArC$\underline{H_2}$C$\underline{H_2}$N), 3.82 (t, J = 5.8 Hz, 2H, OC$\underline{H_2}$), 3.87 (s, 2H, ArC$\underline{H_2}$N), 4.30 (t, J = 6.6 Hz, 2H, NC$\underline{H_2}$CH$_2$CH$_2$O), 6.78 (d, J = 9.0 Hz, 2H, Ar-H), 7.23 (d, J = 9.0 Hz, 2H, Ar-H), 7.35 (s, 2H, Ar-H), 7.67 (s, 1H, Ar-H). MS (ESI, positive) m/z calcd for $C_{22}H_{23}ClF_3N_2O$ (M+H): 423.15; found 423.05. Anal. calcd. for $C_{22}H_{22}ClF_3N_2O$: C, 62.49; H, 5.24; N, 6.62. Found: C, 62.48; H, 5.23; N, 6.63. HPLC purity: 96.8%.

8-chloro-5-(3-(4-chlorophenoxy)propyl)-2-methyl-2,3,4,5-tetrahydro-1*H*-pyrido[4,3-*b*]indole (C14). Yellow oil, 0.16 g (55.9%). ^{1}H-NMR ($CDCl_3$, 600 MHz) δ: 2.18 (m, 2H, NCH$_2$C$\underline{H_2}$CH$_2$O), 2.57 (s, 3H, NC$\underline{H_3}$), 2.84 (m, 4H, ArC$\underline{H_2}$C$\underline{H_2}$N), 3.69 (s, 2H, ArC$\underline{H_2}$N), 3.81 (t, J = 6.0 Hz, 2H, NC$\underline{H_2}$CH$_2$CH$_2$O), 4.23 (t, J = 6.6 Hz, 2H, OC$\underline{H_2}$), 6.77-6.79 (d, J = 9.0 Hz, 2H, Ar-H), 7.03(dd, J_1 = 1.8 Hz, J_2 = 8.0 Hz, 1H, Ar-H), 7.17 (d, J = 8.0 Hz, 1H, Ar-H), 7.22-7.23 (d, J = 9.0 Hz, 2H, Ar-H), 7.35 (d, 1H, J = 1.8 Hz, Ar-H). MS (ESI, positive) m/z calcd for $C_{21}H_{23}Cl_2N_2O$ (M+H): 389.12; found 389.04. Anal. calcd. for $C_{21}H_{22}Cl_2N_2O$: C, 64.79; H, 5.70; N, 7.20. Found: C, 64.78; H, 5.68; N, 7.21. HPLC purity: 95.3%.

5-(3-(4-chlorophenoxy)propyl)-8-methoxy-2-methyl-2,3,4,5-tetrahydro-1*H*-pyrid

o[4,3-*b*]indole (C15). Yellow oil, 0.19 g (66.7%). ^{1}H-NMR ($CDCl_3$, 600 MHz) δ: 2.18 (m, 2H, $NCH_2C\underline{H_2}CH_2O$), 2.54 (s, 3H, $NC\underline{H_3}$), 2.78-2.81 (m, 4H, $ArC\underline{H_2}C\underline{H_2}N$), 3.64 (s, 2H, $ArC\underline{H_2}N$), 3.80 (t, J = 5.7 Hz, 2H, $OC\underline{H_2}$), 3.82 (s, 3H, $OC\underline{H_3}$), 4.20 (t, J = 6.6 Hz, 2H, $NC\underline{H_2}CH_2CH_2O$), 6.73 (dd, J_1 = 1.8 Hz, J_2 = 8.0 Hz, 1H, Ar-H), 6.76-6.77 (d, J = 9.0 Hz, 2H, Ar-H), 6.85 (d, J = 1.8 Hz, 1H, Ar-H), 7.14 (d, J = 8.0 Hz, 1H, Ar-H), 7.18-7.21 (d, J = 9.0 Hz, 2H, Ar-H). ^{13}C-NMR ($CDCl_3$, 75 MHz) δ: 20.76, 29.67, 39.74, 43.45, 51.29, 51.69, 56.03, 64.40, 100.14, 110.19, 111.67, 115.79 (2C), 125.58, 126.03, 129.65 (2C), 129.73, 131.77, 131.88, 154.30, 157.10. MS (ESI, positive) m/z calcd for $C_{22}H_{26}ClN_2O_2$ (M+H): 385.17; found 385.14. Anal. calcd. for $C_{22}H_{25}ClN_2O_2$: C, 68.65; H, 6.55; N, 7.28. Found: C, 68.64; H, 6.54; N, 7.29. HPLC purity: 97.2%.

6,8-dichloro-5-(3-(4-chlorophenoxy)propyl)-2-methyl-2,3,4,5-tetrahydro-1*H*-pyri do[4,3-*b*]indole (C16). Yellow solid, 0.10 g (55.3%). ^{1}H-NMR ($CDCl_3$, 600 MHz) δ: 2.21 (m, 2H, $NCH_2C\underline{H_2}CH_2O$), 2.52 (s, 3H, $-C\underline{H_3}$), 2.75 (t, J = 5.4 Hz, 2H, $ArCH_2C\underline{H_2}N$), 2.81 (t, J = 5.4 Hz, 2H, $ArC\underline{H_2}CH_2N$), 3.56 (s, 2H, $ArC\underline{H_2}N$), 3.88 (t, J = 5.8 Hz, 2H, $NC\underline{H_2}CH_2CH_2O$), 4.53 (t, J = 7.2 Hz, 2H, $OC\underline{H_2}$), 6.76-6.79 (d, 2H, Ar-H), 7.08 (d, J = 1.8 Hz, 1H, Ar-H), 7.21-7.24 (d, 2H, Ar-H), 7.24 (d, J = 1.8 Hz, 1H, Ar-H). MS (ESI, positive) m/z calcd for $C_{21}H_{22}Cl_3N_2O$ (M+H): 423.08; found 423.12. Anal. calcd. for $C_{21}H_{21}Cl_3N_2O$: C, 59.52; H, 4.99; N, 6.61. Found: C, 59.52; H, 4.98; N, 6.60. HPLC purity: 98.2%.

8-chloro-5-(3-(4-chlorophenoxy)propyl)-2-ethyl-2,3,4,5-tetrahydro-1*H*-pyrido[4,3 -*b*]indole (C17). Yellow oil, 0.21 g (69.1%). ^{1}H-NMR ($CDCl_3$, 600 MHz) δ: 1.23 (t,

3H, J = 7.2 Hz, C$\underline{H_2}$CH_3), 2.18 (m, 2H, -NCH_2C$\underline{H_2}$CH_2O-), 2.69 (q, J = 7.2 Hz, 2H, NC$\underline{H_2}$CH_3), 2.85 (m, 4H, ArC$\underline{H_2}$C$\underline{H_2}$N), 3.69 (s, 2H, ArC$\underline{H_2}$N), 3.81 (t, J = 6.0 Hz, 2H, NC$\underline{H_2}$CH_2CH_2O), 4.21 (t, J = 6.6 Hz, 2H, OC$\underline{H_2}$), 6.77-6.79 (d, 2H, Ar-H), 7.02 (dd, J_1 = 1.8 Hz, J_2 = 8.0 Hz, 1H, Ar-H), 7.16 (d, J = 8.0 Hz, 1H, Ar-H), 7.22-7.24 (d, 2H, Ar-H), 7.36 (d, J = 1.8 Hz, 1H, Ar-H). ^{13}C-NMR ($CDCl_3$, 125 MHz) δ: 12.54, 22.82, 29.74, 39.49, 48.92, 50.18, 51.68, 64.04, 107.71, 109.82, 115.71, 117.22, 120.94, 124.73, 125.89, 126.81, 129.41, 134.96, 157.06. MS (ESI, positive) m/z calcd for $C_{22}H_{25}Cl_2N_2O$ (M+H): 403.13; found 403.11. Anal. calcd. for $C_{22}H_{24}Cl_2N_2O$: C, 65.51; H, 6.00; N, 6.95. Found: C, 65.50; H, 6.01; N, 6.96. HPLC purity: 98.2%.

8-chloro-5-(3-(4-chlorophenoxy)propyl)-2-propyl-2,3,4,5-tetrahydro-1*H*-pyrido[4,3-*b*]indole (C18). Yellow oil, 0.18 g (55.9%). ^{1}H-NMR ($CDCl_3$, 600 MHz) δ: 0.96 (t, 3H, J = 7.2 Hz, C$\underline{H_2}$CH_3), 1.66 (m, 2H, NCH_2C$\underline{H_2}$CH_3), 2.18 (m, 2H, NCH_2C$\underline{H_2}$CH_2O), 2.57 (m, 2H, NC$\underline{H_2}$CH_2CH_3), 2.83 (m, 4H, ArC$\underline{H_2}$C$\underline{H_2}$N), 3.68 (s, 2H, ArC$\underline{H_2}$N), 3.81 (t, J = 5.7 Hz, 2H, NC$\underline{H_2}$CH_2CH_2O), 4.22 (t, J = 6.6 Hz, 2H, OC$\underline{H_2}$), 6.78 (d, 2H, Ar-H), 7.03 (dd, J_1 = 1.8 Hz, J_2 = 8.0 Hz, 1H, Ar-H), 7.16 (d, J = 8.0 Hz, 1H, Ar-H), 7.23 (d, 2H, Ar-H), 7.36 (d, J = 1.8 Hz, 1H, Ar-H). ^{13}C-NMR ($CDCl_3$, 75 MHz) δ: 12.10, 20.79, 22.97, 29.87, 39.59, 49.56, 50.65, 60.09, 64.53, 108.06, 109.93, 115.82 (2C), 117.34, 121.00, 124.80, 126.00, 126.98, 129.54 (2C), 135.04, 135.22, 157.20. MS (ESI, positive) m/z calcd for $C_{23}H_{27}Cl_2N_2O$ (M+H): 417.15; found 417.09. Anal. calcd. for $C_{23}H_{26}Cl_2N_2O$: C, 66.19; H, 6.28; N, 6.71. Found: C, 66.18; H, 6.29; N, 6.72. HPLC purity: 98.5%.

4-fluoro-N-(3-(8-fluoro-3,4-dihydro-1*H*-pyrido[4,3-*b*]indol-5(2*H*)-yl)propyl)anilin

e (C19). Pale solid, 0.08 g (50.1%). ^{1}H-NMR (500 Hz, $CDCl_3$) δ: 1.92 (m, 2 H, -$NCH_2C\underline{H_2}CH_2NH$-), 2.93 (t, 2 H, $ArC\underline{H_2}CH_2N$), 3.07 (t, 2 H, $ArCH_2C\underline{H_2}N$), 3.46 (t, 2 H, -$NCH_2CH_2C\underline{H_2}NH$-), 4.23 (t, *J* = 7.0 Hz, 2 H, -$NC\underline{H_2}CH_2CH_2NH$-), 4.27 (s, 2 H, $ArC\underline{H_2}N$), 6.51～7.53 (m, 7 H, Ar-H). MS (ESI, positive) m/z calcd for $C_{20}H_{22}F_2N_3$ (M+H): 342.18; found 342.21. Anal. calcd. for $C_{20}H_{21}F_2N_3$: C, 70.36; H, 6.20; N, 12.31. Found: C, 70.35; H, 6.20; N, 12.32. HPLC purity: 95.4%.

8-fluoro-5-(3-(4-fluorophenoxy)propyl)-2,3,4,5-tetrahydro-1*H*-pyrido[4,3-*b*]indol e (C20). Brown solid, 0.12 g (59.6%). ^{1}H-NMR (500 Hz, $CDCl_3$) δ: 2.08 (m, 2 H, -$NCH_2\underline{CH_2}CH_2O$-), 2.85 (t, 2 H, $ArC\underline{H_2}CH_2N$), 3.21 (t, 2 H, $ArCH_2C\underline{H_2}N$), 3.86 (t, *J* = 6.6 Hz, 2 H, -$NCH_2CH_2C\underline{H_2}O$-), 4.03 (s, 2 H, $ArC\underline{H_2}N$), 4.25 (t, *J* = 5.7 Hz, 2 H, -$NC\underline{H_2}CH_2CH_2O$-), 6.89～7.44 (m, 7 H, Ar-H). MS (ESI, positive) m/z calcd for $C_{20}H_{21}F_2N_2O$ (M+H): 343.16; found 343.22. Anal. calcd. for $C_{20}H_{20}F_2N_2O$: C, 70.16; H, 5.89; N, 8.18. Found: C, 70.15; H, 5.90; N, 8.17. HPLC purity: 98.1%.

8-fluoro-5-(4-(4-fluorophenoxy)butyl)-2,3,4,5-tetrahydro-1*H*-pyrido[4,3-*b*]indole (C21). Brown solid, 0.10 g (62.4%). ^{1}H-NMR (500 Hz, $CDCl_3$) δ: 1.77 (m, 2H, -$NCH_2CH_2C\underline{H_2}CH_2O$-), 1.93 (m, 2H, -$NCH_2C\underline{H_2}CH_2CH_2O$-), 3.15 (t, *J* = 4.8 Hz, 2H, $ArC\underline{H_2}CH_2N$), 3.56 (t, *J* = 5.8 Hz, 2H, $ArCH_2C\underline{H_2}N$), 3.88 (t, *J* = 6.0 Hz, 2H, -$NCH_2CH_2CH_2C\underline{H_2}O$-), 4.08 (t, *J* = 7.0 Hz, 2H, -$NC\underline{H_2}CH_2CH_2CH_2O$-), 4.37 (s, 2H, $ArC\underline{H_2}N$), 6.79～7.26 (m, 7H, Ar-H). MS (ESI, positive) m/z calcd for $C_{21}H_{23}F_2N_2O$ (M+H): 357.18; found 357.10. Anal. calcd. for $C_{21}H_{22}F_2N_2O$: C, 70.77; H, 6.22; N, 7.86. Found: C, 70.78; H, 6.22; N, 7.87. HPLC purity: 96.4%.

8-fluoro-5-(5-(4-fluorophenoxy)pentyl)-2,3,4,5-tetrahydro-1*H*-pyrido[4,3-*b*]indol

e (C22). Brown solid, 0.09 g (55.8%). ^{1}H-NMR (500 Hz, $CDCl_3$) δ: 1.46 (m, 2H, -$NCH_2CH_2C\underline{H_2}CH_2CH_2O$-), 1.76 (m, 4H, -$NCH_2C\underline{H_2}CH_2C\underline{H_2}CH_2O$-), 3.13 (t, *J* = 3.5 Hz, 2H, $ArC\underline{H_2}CH_2N$), 3.56 (t, *J* = 5.3 Hz, 2H, $ArCH_2C\underline{H_2}N$), 3.86 (t, *J* = 6.3 Hz, 2H, -$NCH_2CH_2CH_2CH_2C\underline{H_2}O$-), 4.00 (t, *J* = 7.3 Hz, 2H, -$NC\underline{H_2}CH_2CH_2CH_2CH_2O$-), 4.36 (s, 2H, $ArC\underline{H_2}N$), 6.79～7.26 (m, 7H, Ar-H). MS (ESI, positive) m/z calcd for $C_{22}H_{25}F_2N_2O$ (M+H): 371.19; found 371.24. Anal. calcd. for $C_{22}H_{24}F_2N_2O$: C, 71.33; H, 6.53; N, 7.56. Found: C, 71.34; H, 6.55; N, 7.56. HPLC purity: 96.9%.

8-chloro-5-(3-(4-fluorophenoxy)propyl)-2,3,4,5-tetrahydro-1*H*-pyrido[4,3-*b*]indol e (C23). Brown solid, 0.15 g (62.4%). ^{1}H-NMR (500 Hz, $CDCl_3$) δ: 2.18 (m, 2H, -$NCH_2C\underline{H_2}CH_2O$-), 2.92 (t, *J* = 5.5 Hz, 2H, $ArC\underline{H_2}CH_2N$), 3.31 (t, *J* = 5.8 Hz, 2H, $ArCH_2C\underline{H_2}N$), 3.79 (t, *J* = 5.6 Hz, 2H, -$NCH_2CH_2C\underline{H_2}O$-), 4.18 (s, 2H, $ArC\underline{H_2}N$), 4.23 (t, *J* = 6.6 Hz, 2H, -$NC\underline{H_2}CH_2CH_2O$-), 6.79～7.36 (m, 7H, Ar-H). MS (ESI, positive) m/z calcd for $C_{20}H_{21}ClFN_2O$ (M+H): 359.13; found 359.22. Anal. calcd. for $C_{20}H_{20}ClFN_2O$: C, 66.94; H, 5.62; N, 7.81. Found: C, 66.95; H, 5.63; N, 7.81. HPLC purity: 97.3%.

5-(3-(3-bromophenoxy)propyl)-8-chloro-2,3,4,5-tetrahydro-1*H*-pyrido[4,3-*b*]indo le (C24). Pale solid, 0.11 g (64.2%). ^{1}H-NMR (500 Hz, $CDCl_3$) δ: 2.18 (m, 2H, -$NCH_2C\underline{H_2}CH_2O$-), 3.12 (t, *J* = 5.5 Hz, 2H, $ArC\underline{H_2}CH_2N$), 3.45 (t, *J* = 5.9 Hz, 2H, $ArCH_2C\underline{H_2}N$), 3.79 (t, *J* = 5.4 Hz, 2H, -$NCH_2CH_2C\underline{H_2}O$-), 4.22 (t, *J* = 6.6 Hz, 2H, -$NC\underline{H_2}CH_2CH_2O$-), 4.35 (s, 2H, $ArC\underline{H_2}N$), 6.79～7.26 (m, 7H, Ar-H). ^{13}C-NMR (150 Hz, DMSO-*d6*) δ: 19.07, 29.03, 39.49, 40.01, 40.56, 64.79, 101.91, 111.20, 113.89, 117.34, 117.49, 121.17, 122.03, 123.56, 123.92, 125.69, 131.18, 133.52, 134.48,

159.19. MS (ESI, positive) m/z calcd for $C_{20}H_{21}BrClN_2O$ (M+H): 419.05; found 419.12. Anal. calcd. for $C_{20}H_{20}BrClN_2O$: C, 57.23; H, 4.80; N, 6.67. Found: C, 57.24; H, 4.80; N, 6.67. HPLC purity: 95.7%.

8-bromo-5-(3-(4-fluorophenoxy)propyl)-2,3,4,5-tetrahydro-1*H*-pyrido[4,3-*b*]indole (C25). Pale solid, 0.11 g (52.8%). ^{1}H-NMR (500 Hz, $CDCl_3$) δ: 2.18 (m, 2H, -$NCH_2C\underline{H_2}CH_2O$-), 2.95 (t, *J* = 5.5 Hz, 2H, $ArC\underline{H_2}CH_2N$), 3.32 (t, *J* = 5.9 Hz, 2H, $ArCH_2C\underline{H_2}N$), 3.78 (t, *J* = 5.5 Hz, 2H, -$NCH_2CH_2C\underline{H_2}O$-), 4.19 (s, 2H, $ArC\underline{H_2}N$), 4.23 (t, *J* = 6.6 Hz, 2H, -$NC\underline{H_2}CH_2CH_2O$-), 6.79～7.26 (m, 7H, Ar-H). MS (ESI, positive) m/z calcd for $C_{20}H_{21}BrFN_2O$ (M+H): 403.08; found 403.11. Anal. calcd. for $C_{20}H_{20}BrFN_2O$: C, 59.56; H, 5.00; N, 6.95. Found: C, 59.55; H, 5.01; N, 6.94. HPLC purity: 95.6%.

5-(3-(4-fluorophenoxy)propyl)-8-methyl-2,3,4,5-tetrahydro-1*H*-pyrido[4,3-*b*]indole (C26). Brown solid, 0.09 g (50.8%). ^{1}H-NMR (500 Hz, $CDCl_3$) δ: 2.19 (m, 2H, -$NCH_2C\underline{H_2}CH_2O$-), 2.43 (s, 3H, -$C\underline{H_3}$), 2.91 (t, 2H, $ArC\underline{H_2}CH_2N$), 3.31 (t, 2H, $ArCH_2C\underline{H_2}N$), 3.79 (t, *J* = 5.6 Hz, 2H, -$NCH_2CH_2C\underline{H_2}O$-), 4.20 (s, 2H, $ArC\underline{H_2}N$), 4.22 (t, *J* = 6.6 Hz, 2H, -$NC\underline{H_2}CH_2CH_2O$-), 6.79～7.20 (m, 7H, Ar-H). MS (ESI, positive) m/z calcd for $C_{21}H_{24}FN_2O$ (M+H): 339.19; found 339.26. Anal. calcd. for $C_{21}H_{23}FN_2O$: C, 74.53; H, 6.85; N, 8.28. Found: C, 74.55; H, 6.85; N, 8.28. HPLC purity: 98.2%.

5-(3-(3-bromophenoxy)propyl)-8-methyl-2,3,4,5-tetrahydro-1*H*-pyrido[4,3-*b*]indole (C27). Brown solid, 0.12 g (55.6%). ^{1}H-NMR (500 Hz, $CDCl_3$) δ: 1.93 (s, 3H, -CH_3), 2.20 (m, 2H, -$NCH_2C\underline{H_2}CH_2O$-), 2.98 (t, 2H, $ArC\underline{H_2}CH_2N$), 3.38 (t, *J* = 5.9 Hz,

2H, $ArCH_2C\underline{H_2}N$), 3.75 (t, 2H, $-NCH_2CH_2C\underline{H_2}O-$), 4.22 (t, *J* = 6.6 Hz, 2H, $-NC\underline{H_2}CH_2CH_2O-$), 4.26 (s, 2H, $ArC\underline{H_2}N$), 6.80～7.20 (m, 7H, Ar-H). MS (ESI, positive) m/z calcd for $C_{21}H_{24}BrN_2O$ (M+H): 399.11; found 399.31. Anal. calcd. for $C_{21}H_{23}BrN_2O$: C, 63.16; H, 5.81; N, 7.02. Found: C, 63.15; H, 5.80; N, 7.02. HPLC purity: 96.6%.

8-fluoro-5-(3-phenoxypropyl)-2,3,4,5-tetrahydro-1*H*-pyrido[4,3-*b*]indole (C28). Brown solid, 0.14 g (66.2%). ^{1}H-NMR (500 Hz, $CDCl_3$) δ: 2.19 (m, 2 H, $-NCH_2\underline{CH_2}CH_2O-$), 2.83 (t, *J* = 5.6 Hz, 2H, $ArC\underline{H_2}CH_2N$), 3.24 (t, *J* = 5.7 Hz, 2H, $ArCH_2C\underline{H_2}N$), 3.84 (t, *J* = 6.6 Hz, 2H, $-NCH_2CH_2C\underline{H_2}O-$), 4.10 (s, 2H, $ArC\underline{H_2}N$), 4.23 (t, 2H, *J* = 5.5 Hz, $-NC\underline{H_2}CH_2CH_2O-$), 6.85～7.30 (m, 8H, Ar-H). MS (ESI, positive) m/z calcd for $C_{20}H_{22}FN_2O$ (M+H): 325.17; found 325.09. Anal. calcd. for $C_{20}H_{21}FN_2O$: C, 74.05; H, 6.53; N, 8.64. Found: C, 74.06; H, 6.53; N, 8.64. HPLC purity: 97.4%.

8-fluoro-5-(3-(4-methoxyphenoxy)propyl)-2,3,4,5-tetrahydro-1*H*-pyrido[4,3-*b*]in dole (C29). Pale solid, 0.16 g (55.4%). ^{1}H-NMR (500 Hz, $CDCl_3$) δ: 2.16 (m, 2H, $-NCH_2C\underline{H_2}CH_2O-$), 2.74 (t, 2H, $ArC\underline{H_2}CH_2N$), 3.19 (t, 2 H, $ArCH_2C\underline{H_2}N$), 3.77 (s, 3H, $-OC\underline{H_3}$), 3.79 (t, *J* = 6.6 Hz, 2H, $-NCH_2CH_2C\underline{H_2}O-$), 4.03 (s, 2H, $ArC\underline{H_2}N$), 4.22 (t, *J* = 5.6 Hz, 2H, $-NC\underline{H_2}CH_2CH_2O-$), 6.78～7.19 (m, 7H, Ar-H). MS (ESI, positive) m/z calcd for $C_{21}H_{24}FN_2O_2$ (M+H): 355.18; found 355.23. Anal. calcd. for $C_{21}H_{23}FN_2O_2$: C, 71.17; H, 6.54; N, 7.90. Found: C, 71.18; H, 6.53; N, 7.91. HPLC purity: 97.3%.

5-(3-(2,5-difluorophenoxy)propyl)-8-fluoro-2,3,4,5-tetrahydro-1*H*-pyrido[4,3-*b*]in dole (C30). Pale solid, 0.12 g (60.8%). ^{1}H-NMR (500 Hz, $CDCl_3$) δ: 2.22 (m, 2H,

-$NCH_2C\underline{H_2}CH_2O$-), 3.18 (t, 2H, $ArC\underline{H_2}CH_2N$), 3.49 (t, 2H, $ArCH_2C\underline{H_2}N$), 3.83 (t, *J* = 6.2 Hz, 2H, $NCH_2CH_2C\underline{H_2}O$), 4.27 (t, *J* = 5.2 Hz, 2H, $NC\underline{H_2}CH_2CH_2O$), 4.37 (s, 2H, $ArC\underline{H_2}N$), 6.57～7.26 (m, 6H, Ar-H). MS (ESI, positive) m/z calcd for $C_{20}H_{20}F_3N_2O$ (M+H): 361.15; found 361.21. Anal. calcd. for $C_{20}H_{19}F_3N_2O$: C, 66.66; H, 5.31; N, 7.77. Found: C, 66.68; H, 5.32; N, 7.78. HPLC purity: 96.9%.

5-(3-(4-bromophenoxy)propyl)-8-fluoro-2,3,4,5-tetrahydro-1*H*-pyrido[4,3-*b*]indole (C31). Brown solid, 0.13 g (63.4%). ^{1}H-NMR (500 Hz, $CDCl_3$) δ: 2.19 (m, 2H, -$NCH_2C\underline{H_2}CH_2O$-), 2.95 (t, 2H, $ArC\underline{H_2}CH_2N$), 3.35 (t, *J* = 5.9 Hz, 2H, $ArCH_2C\underline{H_2}N$), 3.81 (t, *J* = 6.6 Hz, 2H, -$NCH_2CH_2C\underline{H_2}O$-), 4.21 (s, 2H, $ArC\underline{H_2}N$), 4.23 (t, *J* = 5.5 Hz, 2H, -$NC\underline{H_2}CH_2CH_2O$-), 6.73～7.38 (m, 7H, Ar-H). MS (ESI, positive) m/z calcd for $C_{20}H_{21}BrFN_2O$ (M+H): 403.08; found 403.23. Anal. calcd. for $C_{20}H_{20}BrFN_2O$: C, 59.56; H, 5.00; N, 6.95. Found: C, 59.57; H, 5.01; N, 6.96. HPLC purity: 95.8%.

5-(3-(4-(tert-butyl)phenoxy)propyl)-8-fluoro-2,3,4,5-tetrahydro-1*H*-pyrido[4,3-*b*]indole (C32). Brown solid, 0.13 g (52.2%). ^{1}H-NMR (500 Hz, $CDCl_3$) δ: 1.29 (s, 9H, -$C(CH_3)_3$), 2.19 (m, 2H, -$NCH_2\underline{CH_2}CH_2O$-), 2.86 (t, *J* = 5.5 Hz, 2H, $ArC\underline{H_2}CH_2N$), 3.26 (t, *J* = 5.8 Hz, 2H, $ArCH_2C\underline{H_2}N$), 3.84 (t, *J* = 6.7 Hz, 2H, -$NCH_2CH_2C\underline{H_2}O$-), 4.12 (s, 2H, $ArC\underline{H_2}N$), 4.23 (t, *J* = 5.5 Hz, 2 H, -$NC\underline{H_2}CH_2CH_2O$-), 6.79～7.30 (m, 7H, Ar-H). MS (ESI, positive) m/z calcd for $C_{24}H_{30}FN_2O$ (M+H): 381.23; found 381.32. Anal. calcd. for $C_{24}H_{29}FN_2O$: C, 75.76; H, 7.68; N, 7.36. Found: C, 75.77; H, 7.68; N, 7.37. HPLC purity: 95.6%.

8-fluoro-5-(3-(m-tolyloxy)propyl)-2,3,4,5-tetrahydro-1*H*-pyrido[4,3-*b*]indole (C33). Brown solid, 0.08 g (55.1%). ^{1}H-NMR (500 Hz, $CDCl_3$) δ: 2.19 (m, 2H,

-$NCH_2C\underline{H_2}CH_2O$-), 2.32 (s, 3H, -$C\underline{H_3}$), 2.89 (t, *J* = 5.6 Hz, 2H, $ArC\underline{H_2}CH_2N$), 3.28 (t, *J* = 5.7 Hz, 2H, $ArCH_2C\underline{H_2}N$), 3.83 (t, *J* = 6.6 Hz, 2H, -$NCH_2CH_2C\underline{H_2}O$-), 4.15 (s, 2H, $ArC\underline{H_2}N$), 4.23 (t, *J* = 5.5 Hz, 2H, -$NC\underline{H_2}CH_2CH_2O$-), 6.67～7.26 (m, 7H, Ar-H). MS (ESI, positive) m/z calcd for $C_{21}H_{24}FN_2O$ (M+H): 339.19; found 339.21. Anal. calcd. for $C_{21}H_{23}FN_2O$: C, 74.53; H, 6.85; N, 8.28. Found: C, 74.52; H, 6.85; N, 8.27. HPLC purity: 98.3%.

8-fluoro-5-(3-(p-tolyloxy)propyl)-2,3,4,5-tetrahydro-1*H*-pyrido[4,3-*b*]indole (C34). Brown solid, 0.11 g (60.2%). ^{1}H-NMR (500 Hz, $CDCl_3$) δ: 2.13 (m, 2H, -$NCH_2C\underline{H_2}CH_2O$-), 2.27 (s, 3H, -$C\underline{H_3}$), 3.04 (br, 2H, $ArC\underline{H_2}CH_2N$), 3.34 (br, 2H, $ArCH_2C\underline{H_2}N$), 3.75 (t, *J* = 5.5 Hz, 2H, -$NCH_2CH_2C\underline{H_2}O$-), 4.17 (t, *J* = 6.5 Hz, 2H, -$NC\underline{H_2}CH_2CH_2O$-), 4.26 (s, 2 H, $ArC\underline{H_2}N$), 6.73 (d, *J* = 8.6 Hz, 2H), 6.87 (dt, *J* = 2.4 Hz, 9.0 Hz, 1H), 6.97 (dd, *J* = 2.5 Hz, 9.2 Hz, 1H), 7.05 (d, *J* = 8.6 Hz, 2H), 7.19 (dd, *J* = 4.1 Hz, 8.9 Hz, 1H). MS (ESI, positive) m/z calcd for $C_{21}H_{24}FN_2O$ (M+H): 339.19; found 339.25. Anal. calcd. for $C_{21}H_{23}FN_2O$: C, 74.53; H, 6.85; N, 8.28. Found: C, 74.53; H, 6.85; N, 8.27. HPLC purity: 97.6%.

5-(3-(2-chlorophenoxy)propyl)-8-fluoro-2,3,4,5-tetrahydro-1*H*-pyrido[4,3-*b*]indole (C35). Pale solid, 0.11 g (66.9%). ^{1}H-NMR (500 Hz, $CDCl_3$) δ: 2.06 (m, 2H, -$NCH_2C\underline{H_2}CH_2O$-), 3.11 (t, 2H, $ArC\underline{H_2}CH_2N$), 3.52 (t, 2H, $ArCH_2C\underline{H_2}N$), 3.88 (t, 2H, -$NCH_2CH_2C\underline{H_2}O$-), 4.33 (s, 2H, $ArC\underline{H_2}N$), 4.39 (t, 2H, -$NC\underline{H_2}CH_2CH_2O$-), 6.80～7.39 (m, 7H, Ar-H). MS (ESI, positive) m/z calcd for $C_{20}H_{21}ClFN_2O$ (M+H): 359.13; found 359.25. Anal. calcd. for $C_{20}H_{20}ClFN_2O$: C, 66.94; H, 5.62; N, 7.81. Found: C, 66.95; H, 5.63; N, 7.82. HPLC purity: 96.8%.

8-fluoro-5-(3-(o-tolyloxy)propyl)-2,3,4,5-tetrahydro-1*H*-pyrido[4,3-*b*]indole (C36). Pale solid, 0.16 g (70.2%). ^{1}H-NMR (500 Hz, $CDCl_3$) δ: 2.20 (m, 2H, -$NCH_2C\underline{H_2}CH_2O$-), 2.29 (s, 3H, -$C\underline{H_3}$), 3.11 (t, 2H, $ArC\underline{H_2}CH_2N$), 3.40 (t, 2H, $ArCH_2C\underline{H_2}N$), 3.86 (t, *J* = 6.7 Hz, 2H, -$NCH_2CH_2C\underline{H_2}O$-), 4.26 (t, *J* = 5.3 Hz, 2H, -$NC\underline{H_2}CH_2CH_2O$-), 4.30 (s, 2H, $ArC\underline{H_2}N$), 6.70～7.26 (m, 7H, Ar-H). MS (ESI, positive) m/z calcd for $C_{21}H_{24}FN_2O$ (M+H): 339.19; found 339.23. Anal. calcd. for $C_{21}H_{23}FN_2O$: C, 74.53; H, 6.85; N, 8.28. Found: C, 74.55; H, 6.86; N, 8.27. HPLC purity: 98.2%.

5-(3-(3-bromophenoxy)propyl)-8-fluoro-2,3,4,5-tetrahydro-1*H*-pyrido[4,3-*b*]indole (C37). Pale solid, 0.12 g (65.6%). ^{1}H-NMR (500 Hz, $CDCl_3$) δ: 2.17 (m, 2H, -$NCH_2C\underline{H_2}CH_2O$-), 3.12 (t, 2H, $ArC\underline{H_2}CH_2N$), 3.45 (t, 2H, $ArCH_2C\underline{H_2}N$), 3.80 (t, *J* = 6.6 Hz, 2H, -$NCH_2CH_2C\underline{H_2}O$-), 4.22 (t, *J* = 5.4 Hz, 2H, -$NC\underline{H_2}CH_2CH_2O$-), 4.35 (s, 2 H, $ArC\underline{H_2}N$), 6.78～7.22 (m, 7H, Ar-H). ^{13}C-NMR (150 Hz, $CDCl_3$) δ: 22.77, 28.20, 29.47, 40.70, 42.56, 67.72, 102.00, 102.03, 102.82, 102.98, 109.01, 109.18, 110.72, 110.78, 113.94, 117.20, 122.04, 123.26, 124.67, 124.74, 131.11, 132.66, 133.60, 156.17, 157.71, 159.60. MS (ESI, positive) m/z calcd for $C_{20}H_{21}BrFN_2O$ (M+H): 403.08; found 403.11. Anal. calcd. for $C_{20}H_{20}BrFN_2O$: C, 59.56; H, 5.00; N, 6.95. Found: C, 59.57; H, 5.00; N, 6.97. HPLC purity: 96.5%.

5-(3-(2,3-difluorophenoxy)propyl)-8-fluoro-2,3,4,5-tetrahydro-1*H*-pyrido[4,3-*b*]indole (C39). Pale solid, 0.13 g (60.3%). ^{1}H-NMR (500 Hz, $CDCl_3$) δ: 2.21 (m, 2H, -$NCH_2C\underline{H_2}CH_2O$-), 3.19 (t, 2H, $ArC\underline{H_2}CH_2N$), 3.50 (t, 2H, $ArCH_2C\underline{H_2}N$), 3.86 (t, *J* = 6.8 Hz, 2H, -$NCH_2CH_2C\underline{H_2}O$-), 4.13 (t, 2H, -$NC\underline{H_2}CH_2CH_2O$-), 4.28 (s, 2H,

ArC$\underline{H_2}$N), 6.8～7.05 (m, 6H, Ar-H). MS (ESI, positive) m/z calcd for $C_{20}H_{20}F_3N_2O$ (M+H): 361.15; found 361.22. Anal. calcd. for $C_{20}H_{19}F_3N_2O$: C, 66.66; H, 5.31; N, 7.77. Found: C, 66.68; H, 5.29; N, 7.77. HPLC purity: 97.0%.

8-fluoro-5-(3-(2,4,6-trifluorophenoxy)propyl)-2,3,4,5-tetrahydro-1*H*-pyrido[4,3-*b*]indole (C40). Brown solid, 0.11 g (59.8%). ^{1}H-NMR (500 Hz, $CDCl_3$) δ: 2.09 (m, 2H, -NCH$_2$C$\underline{H_2}$CH$_2$O-), 3.02 (t, *J* = 5.5 Hz, 2H, ArC$\underline{H_2}$CH$_2$N), 3.42 (t, *J* = 5.8 Hz, 2H, ArCH$_2$C$\underline{H_2}$N), 3.96 (t, *J* = 7.0 Hz, 2H, -NCH$_2$CH$_2$C$\underline{H_2}$O-), 4.22 (s, 2H, ArC$\underline{H_2}$N), 4.31 (t, 2H, -NC$\underline{H_2}$CH$_2$CH$_2$O-), 6.45～7.29 (m, 5H, Ar-H). MS (ESI, positive) m/z calcd for $C_{20}H_{19}F_4N_2O$ (M+H): 379.14; found 379.30. Anal. calcd. for $C_{20}H_{18}F_4N_2O$: C, 63.49; H, 4.80; N, 7.40. Found: C, 63.50; H, 4.81; N, 7.40. HPLC purity: 97.3%.

5-(3-(2,6-difluorophenoxy)propyl)-8-fluoro-2,3,4,5-tetrahydro-1*H*-pyrido[4,3-*b*]indole (C41). Brown solid, 0.13 g (56.6%). ^{1}H-NMR (500 Hz, $CDCl_3$) δ: 2.13 (m, 2H, -NCH$_2$C$\underline{H_2}$CH$_2$O-), 3.12 (t, *J* = 5.5 Hz, 2H, ArC$\underline{H_2}$CH$_2$N), 3.50 (t, *J* = 6.0 Hz, 2H, ArCH$_2$C$\underline{H_2}$N), 4.07 (t, *J* = 7.1 Hz, 2H, -NCH$_2$CH$_2$C$\underline{H_2}$O-), 4.13 (s, 2 H, ArC$\underline{H_2}$N), 4.29 (t, *J* = 5.4 Hz, 2H, -NC$\underline{H_2}$CH$_2$CH$_2$O-), 6.89～7.27 (m, 6 H, Ar-H). MS (ESI, positive) m/z calcd for $C_{20}H_{20}F_3N_2O$ (M+H): 361.15; found 361.16. Anal. calcd. for $C_{20}H_{19}F_3N_2O$: C, 66.66; H, 5.31; N, 7.77. Found: C, 66.67; H, 5.30; N, 7.77. HPLC purity: 98.6%.

8-fluoro-5-(3-(2,3,4-trifluorophenoxy)propyl)-2,3,4,5-tetrahydro-1*H*-pyrido[4,3-*b*]indole (C42). Brown solid, 0.11 g (54.7%). ^{1}H-NMR (500 Hz, $CDCl_3$) δ: 2.19 (m, 2H, -NCH$_2$C$\underline{H_2}$CH$_2$O-), 2.95 (t, *J* = 5.1 Hz, 2H, ArC$\underline{H_2}$CH$_2$N), 3.35 (t, *J* = 5.8 Hz, 2H, ArCH$_2$C$\underline{H_2}$N), 3.85 (t, *J* = 6.7 Hz, 2H, -NCH$_2$CH$_2$C$\underline{H_2}$O-), 4.19 (t, *J* = 5.5 Hz, 2H,

-NC$\underline{H_2}$$CH_2CH_2O$-), 4.21 (s, 2H, ArC$\underline{H_2}$N), 6.48～7.25 (m, 5H, Ar-H). MS (ESI, positive) m/z calcd for $C_{20}H_{19}F_4N_2O$ (M+H): 379.14; found 379.27. Anal. calcd. for $C_{20}H_{18}F_4N_2O$: C, 63.49; H, 4.80; N, 7.40. Found: C, 63.51; H, 4.80; N, 7.40. HPLC purity: 96.2%.

3. Structure-activity relationships

Firstly, the role of side chain attached to the carboline N5 atom was investigated. Compounds **C1-C3** with substituted benzyl groups showed broader antifungal spectrum compared with the lead compound **1**, but their antifungal activity against *C. albicans* and *C. neoformans* was slightly decreased. Interestingly, **C1-C3** also showed moderate inhibitory activity against *A. fumigatus*, while **FLC** was inactive. On the contrary, other substitutions (compounds **C4-C10**), such as benzoyl group, phenylacetyl, or alkyl group, led to almost loss of the antifungal activity, indicating that the side chain attached to the **N5** atom was important for the antifungal activity.

Moreover, compounds **C11-C42** with a longer side chain (a phenoxyalkyl or alkylaniline side chain) were also synthesized. Most of these compounds, particularly **C19-C42**, showed better inhibitory activity and broader antifungal spectrum than compounds **C1-C10** and the lead compound **1**. The results suggested that a flexible and moderate hydrophobic side chain was more favorable for the antifungal activity. Compounds with various substitutions attached to the **N8** atom were also designed and synthesized (compounds **C14**, **C17**, **C18** and **C23**). Among them, compound **C23** showed better activity and broader antifungal spectrum with a MIC_{80} value of 4 μg/mL against all the tested *Candida species.* The introduction of the alkyl

substitutions such as methyl (**C12**), ethyl (**C17**) or propyl (**C18**) on the **N8** atom resulted in obvious decrease of the inhibitory activity. Moreover, the precursor of compound **C23**, with ethoxycarbonyl group at the **N8** atom was totally inactive (data not shown). The SAR on the **N8** atom revealed that free NH was more favorable to enhance the antifungal activity.

Compounds with different substitutions on the aromatic ring of the carboline scaffold were also designed and synthesized (compounds **C11-C16** and **C22-C27**). The electron-withdrawing group such as CF_3 (**C13**) and multi-halogens such as 2,4-2Cl (**C16**) decreased the inhibitory activity, with the MIC_{80} value larger than 64 μg/mL against *C. albicans*. On the contrary, electron-donating group such as methyl (**C12**), methoxyl (**C15**) and single halogen (compounds **C22-C25**) could slightly enhance the antifungal activity. Among them, compounds **C23** and **C25** showed good antifungal activity against all tested *Candida species* with MIC_{80} values of 4 μg/mL, which were better than the lead compound **1**. Moreover, they showed comparable inhibitory activity against *C. krusei* compared with **FLC** (MIC_{80} = 4 μg/mL).

The antifungal activity of compounds **C20-C22** with different length of alkyl side chain was investigated. Among them, compound **C20**, with a propyl side chain, showed the best antifungal activity. Replacement of the phenoxyalkyl group (**C20**) by the alkylaniline side chain (**C19**) led to slight decrease of the antifungal activity, indicating the oxygen atom in the side chain was more favorable than the nitrogen atom. The effect of various substitutions on the terminal phenyl group of the side chain was also investigated (compounds **C28-C42**). Compound **C38** with 4-Cl

substitution showed potent inhibitory activity against all the tested fungal pathogens (MIC_{80} range: 1 μg/mL to 4 μg/mL). It showed the best antifungal activity against *C. albicans* and *T. rubrum* with MIC_{80} values of 2 μg/mL. Moreover, compound **C38** also showed potent inhibitory activity against *C. neoformans* and *M. gypseum* (MIC_{80} = 1 μg/mL), which was superior or comparable to **FLC**. For the *C. krusei* strain, compound **C38** also showed comparable inhibitory activity to **FLC** with a MIC_{80} value of 4 μg/mL. In contrast, compound **C35** with 2-Cl substitution showed obviously decreased antifungal activity (*C. albicans*, MIC_{80} = 32 μg/mL). The 3-Br substituted derivative **C37** also showed potent inhibitory activity against *C. albicans* and *M. gypseum* (MIC_{80} = 2 μg/mL). Moreover, its inhibitory activity against *C. krusei* (MIC_{80} = 2 μg/mL) and *T. rubrum* (MIC_{80} = 2 μg/mL) was comparable to **FLC**. Moreover, compounds **C39-C42** with multi-fluorine substitutions showed slightly increased antifungal activity compared with the 4-F substituted compound **C20**. Other compounds with substitutions such as 4-Br (**C31**), 4-*tert*-butyl (**C32**), 3-CH_3 (**C33**), 4-CH_3 (**C34**), and 2-CH_3 (**C36**) also showed moderate inhibitory activity against *C. albicans* (MIC_{80} = 8 μg/mL). Interestingly, most compounds showed slightly better inhibitory activity against *C. neoformans* than *C. albicans*. Among the synthesized carboline derivatives, the most active compound **C38** represent a promising antifungal lead with novel chemotype, which was subjected to a series of *in vitro* assays.

4. Experimental protocols of biological assays

In vitro antifungal testing

In vitro antifungal activity was measured by the serial dilution method in 96-well

microtest plates [2,3]. Test fungal strains were obtained from the American Type Culture Collection (ATCC) or were clinical isolates. The determination of minimum inhibitory concentration (MIC) was performed according to the recommendations of National Committee for Clinical Laboratory Standards (NCCLS) with RPMI 1640 (Sigma) buffered with 0.165M MOPS (Sigma) as the test medium. The MIC_{80} values were defined as the lowest concentrations of the drugs (alone or in combination) that inhibited fungal growth by 80% compared with that of the drug-free wells. The fractional inhibitory concentration (FIC) index is defined as the sum of the MIC_{80} of each drug when used in combination divided by the MIC_{80} of the drug used alone. Synergy and antagonism were defined by FIC indices of ≤ 0.5 and > 4, respectively. An FIC index result of > 0.5 but ≤ 4 was considered indifferent [4,5]. Test compounds were dissolved in DMSO serially diluted in growth medium. The yeast strains were incubated at 35°C, and the dermatophytes at 28°C. Growth MIC_{80} was determined at 24 h for *Candida spp.*, at 72 h for *C. neoformans*, and at 7 days for *filamentous* fungi.

Time-kill curves for testing fungicidal activity

Time-kill curves were used for testing the fungicidal activity of lead compound **1** and compound **C38**. The experimental procedure was performed according to the method reported by Jiang *et al* [4]. *C. albicans* SC5314 (**FLC** sensitive with $MIC_{80} = 0.5$ μg/mL) and *C. albicans* 103 (**FLC** resistant with $MIC_{80} > 64$μg/mL) in RPMI 1640 medium were prepared respectively at the starting inoculum of 10^5 CFU/mL. The concentration for **FLC** and lead compound **1** were both 32 μg/mL, and a drug-free sample served as a growth control. Concentrations for compound **C38** were 2, 4, 8, 16,

and 32 μg/mL, respectively. DMSO comprised < 1% of the total test volume. At predetermined time points (0, 12, 24, and 48 h after incubation with agitation at 35°C), a 100-μL aliquot was removed from every solution and serially diluted 10-fold in sterile water. A 100-μl aliquot from each dilution was streaked on the Sabouraud dextrose agar plate. Colony counts were determined after incubation at 35°C for 48 h. All time-kill curve experiments were conducted in duplicate, and mean colony count data (log_{10} CFU/mL) were plotted as a function of time for each isolate.

***In vitro* biofilm inhibition assay**

The experimental procedure was performed according to the method reported by Cao et al [6]. Standardized *C. albicans* cells (1.0×10^6 cells/mL in SC medium) were introduced into the wells of 96-well tissue culture plates (Corning Inc., Corning, NY) and incubated at 37°C for the initial 1 h adhesion. After that, the medium was aspirated and non-adherent cells were removed. Fresh RPMI 1640 medium with or without drugs was then added to adherent cells. The concentration for **FLC** was 64 μg/mL, and a drug-free sample served as a control. Concentrations for compound **C38** were 2, 4, 8, 16, 32, and 64 μg/mL, respectively. Then the plates were incubated for a further 48 h at 37°C. A semi-quantitative measure of biofilm formation was calculated by using an XTT [2,3-bis(2-methoxy-4-nitro-5-sulfo-phenyl)-2H-tetrazolium-5-carboxanilide] reduction assay [7]. Briefly, adherent cells were washed twice with PBS and then incubated with 0.5 mg/mL XTT and 10 μM menadione in PBS at 37°C for 90 min. Optical density at 490 nm (OD_{490}) was determined using a microtiter plate reader.

Inhibition of hyphal growth assay

C. albicans SC5314 cells were incubated at 37°C in RPMI 1640 for 16 h in the absence or in the presence of different concentrations of compound **C38** (2 to 8 μg/mL) or **FLC** (8 μg/mL). All cells were viewed by light microscopy at × 400 to assess hyphal formation.

Transmission Electron Microscopy

Transmission electron microscopies of *C. albicans* SC5314 cells were obtained according to the protocol described by Jia et al [8]. In the absence or presence of **FLC** (8 μg/mL) or compound **C38** (8 μg/mL), *C. albicans* SC5314 cells were collected after 8 h of growth in liquid RPMI 1640 medium supplemented with 0.0025% uridine, washed twice with PBS solution, fixed at 48°C for 24 h in 500 mL fixative solution (sodium cacodylate buffer, pH 7.2, containing 4% polyoxymethylene). The samples were then washed with saline and postfixed for 90 min with 1% phosphotungstic acid. The fixed cells were dehydrated through a graded series of ethanol and embedded with EPON-812. Ultrathin sections were prepared and observed after double staining with uranium and lead under a transmission electron microscope (HITACHI H-800, Japan) with 1×10^4 magnification.

GC-MS analysis of sterol composition

Analysis of sterol composition in *C. albicans* SC5314 cells was performed by means of GC-MS, wherein the components of a complex sterol mixture were separated and subsequently analyzed by mass spectrometry. Samples for gas chromatography-mass spectrometry were prepared according to the procedure described by Jia et al [8]. The

sterols in *C. albicans* cells were analyzed using a 7890A GC system (Agilent Technologies). GC-MS data were analyzed using Agilent software, and using NIST Spectrum Database to match the MS data.

Cytochrome P450 enzymes inhibition assay

Material		**Equipment**	
HLM	BD99268	Janus	PerkinElmer
Protein Conc.(mg/mL)	19.2	Mass Spectrometer	Xevo TQ-S，Waters
$MgCl_2$	Sigma	UPLC	UPLC H-Class，Waters
NADPH	Roche		
Tris	Sigma		
BSA	Solarbio		

HLM, human liver microsome

Probe Substrate	**Source**	**Isoform**	**Conc.(μM)**	**Metabolite**
Tolbutamide	Sigma	2C9	50	4-Hydroxy Tolbutamide
S-mephenytoin	Sigma	2C19	10	4′-Hydroxy Mephenytoin
Testosterone	Dr.Ehrenstorfer GmbH	3A4-M	20	6-Hydroxy Testosterone
Midazolam	TRC	3A4-T	2	1′-Hydroxy Midazolam

Positive Inhibitor	**Source**	**Isoform**	**Conc.(μM)**
Sulfaphenazole	Sigma	2C9	2/0.2/0.02
Tranylcypromine	Santa Cruz	2C19	33.3/3.33/0.333
Ketoconazole	TCI	3A4-M	0.1/0.01/0.001
Ketoconazole	TCI	3A4-T	0.1/0.01/0.001

Experiments are performed in 96-well plates with final incubation volume of 100 μL per well. Each well contains 20 μL HLM (final concentration of HLM 0.3 mg/mL) and 50 μL test compound or positive control inhibitor mixture and 20 μL probe substrates in 0.1M Tris (pH 7.4). After pre-incubated at 37°C for 10 min, the reaction

starts with the addition of 10 μL NADPH to make it 1 mM final concentration. Plates are incubated at 37°C for 15 min before reactions are quenched by the addition of 100 μL acetonitrile with a mixture of internal standard (propranolol, nadolol) (50 nM). After reactions are terminated, plates are centrifuged, and supernatants are analyzed by LC/MS/MS.

References

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5. A zoom out figure for the morphology of the whole fungi cell

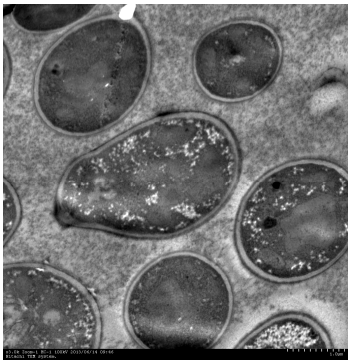

Figure S1. A zoom out figure to show the morphology of the whole fungi cell after treated with compound **C38** at 8 μg/mL.

6. *In vitro* CYP inhibition assessment of positive drugs

Table S1. *In vitro* CYP inhibition assessment of positive inhibitors.

Positive inhibitor	Isoenzyme	% Inhibition			IC_{50} μM	Potential inhibition[a]
		25 μM	2.5 μM	0.25 μM		
Sulfaphenazole	2C9	92	59	10	0.147	High
Tranycypromine	2C19	87	54	17	2.69	high
Ketoconazole	3A4-T	85	28	3	0.0225	High
Ketoconazole	3A4-M	74	16	-2	0.039	High

[a] IC_{50} > 10 μM, CYP inhibition low; 10 μM > IC_{50} > 3 μM, CYP inhibition moderate; 3 μM > IC_{50}, CYP inhibition high.